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CUB - PLS final comment letter BOP. AD C. Smith

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RECEIVED

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SUPERFUND BRANCH

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Site:	Syntex Verona
ID#:	MOD007452154
Break:	6.4
Other:	1-13-87

Dear Dr. Wagoner:

The remedial alternatives reported in conjunction with book 1 of the "final report" submitted to EPA by Syntex Agribusiness, Inc. of the Verona plant were reviewed. This report was received November 24, 1986.

It was difficult to evaluate these data. Therefore additional information such as maps, etc. were requested. Unfortunately, even with this additional information, it was difficult to evaluate the site. It would have been useful to have had a summary of the total number of samples analyzed, the percent of samples above 1 ppb and percent above 50 ppb and the percent above 100 ppb. For each area the ranges and geometric means should also have been given. A simple drawing indicating contaminated areas above 1 ppb, 1-10 ppb, etc. would have made it easier for uninitiated reviewers to evaluate the extent of contamination above various levels. This should be coordinated with the proposed remedial action.

A better description of the potential use of the site is needed. For instance, if it is anticipated that construction will be done at any of these sites concentrations above 50 ppb should definitely be removed. During construction there will be erosion and workers may become contaminated. There may also be tracking. Simply stabilizing contaminated areas above 50 ppb, with top soil and vegetation is insufficient, unless they are in very remote areas.

After reviewing all available data and after additional discussions with EPA personnel in Kansas City, it was determined that the levels in most areas of the Syntex - Agribusiness at the Verona plant in Missouri were either non-detectable or in the low ppb range. Since this is an industrial site which undoubtedly will remain an industrial site, such concentrations are not of concern.

The following statements which are contained in section III of the Syntex document and which claim to follow CDC guidelines and I quote,

"The proposed remedial actions for Verona (contained in Section III. Action Levels infra.) are equivalent to those suggested by CDC for a comparable industrial site. Specifically, for TCDD levels several fold or more above 1 ppb, CDC recommends no remedial action (Kimbrough et al. 1984). Syntex proposes to maintain vegetation to help prevent

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soil erosion in areas with levels of up to 50 ppb. For TCDD levels up to 100 ppb, CDC recommends paving over contaminated areas (Kimbrough et al. 1984). For 50-300 ppb, Syntex proposes a similar action of installing a six inch topsoil cover and revegetating. For the 300-3000 ppb range Syntex proposes installing a 12" topsoil cover and revegetating. For areas with greater than 3000 ppb, Syntex proposes excavating, installing 6" of topsoil, and revegetating."

are either taken out of context or misinterpreted and the remedial actions proposed in the document are only partially acceptable. It is also not clear why in the proposal for remediation levels of 2,3,7,8-TCDD in soil are mentioned for which no measured 2,3,7,8-TCDD concentrations are presented (such as 3000 ppb). Because of these observation there is some concern, that not all analytical results were presented. However, if all results were submitted, then the proposal to stabilize areas with top soil and vegetation, contaminated between non-detectable - 20 ppb levels is acceptable. This would take care of a large portion of the area under discussion.

The lagoon presents a special problem. The degree of contamination has not been sufficiently characterized. In this area the water table is quite high (at times it may be up to 3 feet below the soil surface) and it is not clear what is under the groundwater table. From the available results thus far portions of the "old lagoon" need to be excavated, and the soil properly disposed or decontaminated.

Presently it cannot be estimated how much soil actually needs to be removed. Since after excavation filling the area with clean soil would result in overall dilution of the concentrations of TCDD, it might be possible to leave contaminated soil between 20-50 ppb. Whether this would be possible would depend on further information about the total amount of TCDD in that area and the situation with the water table. For decision making purposes at locations where soil removal is required, the newly exposed surface shall be sampled using EPA's 95 percent confidence limit protocol.

From a human health point of view the concentration of other chemicals found at the site are of no concern. For instance, concentrations of hexachlorophene below 1 percent in soil do not present a health risk for adults. Hexachlorophene was used as a germicide in PhisoHex at a concentration of 3 percent. Because of the toxicity caused in infants when applied to large surface areas of the skin, its use was restricted in the United States and other countries. A review article about the toxicity of hexachlorophene is attached.

The risk calculations in this document were not reviewed in detail. The estimate of soil ingestion by children is too low based on our experience. However, for this site soil ingestion by children is not an issue, since it is an industrial site.

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No attempt was made to review the concentrations of effluents measured in the wells in detail. They consist primarily of priority pollutants and some solvents. It is assumed that none of this water is used for drinking water.

Finally during construction or actual remedial activities such as excavation, when there is a potential for significant dust generation, CDC's recommendations should be followed of requiring workers to wear NIOSH-approved dust masks or properly fit-tested respirators, and other protective clothing, depending on the situation. Implementation of all personal safety measures should be supervised by a qualified industrial hygienist.

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Medical Officer

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Enclosure

cc:

Mark McClanahan

Chapter 4

Hexachlorophene: Toxicity and Use as an Antibacterial Agent

RENATE D. KIMBROUGH

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I. INTRODUCTORY REMARKS

Hexachlorophene [2,2'-methylene-bis-(3,4,6-trichlorophenol)] (Fig. 1) is a bacteriostatic agent that was first synthesized in 1939, patented in

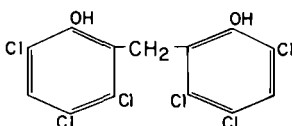


FIG. 1. Hexachlorophene [2,2'-methylene-bis-(3,4,6-trichlorophenol)].

1941, and introduced commercially after World War II (1). The patent of the Swiss-based firm Givaudan expired several years ago and with it the control the company had had over this product (2).

Hexachlorophene, like the herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), is made from 2,4,5-trichlorophenol by condensing two molecules with formaldehyde in the presence of sulfuric acid or some other strong catalyst (1). Before the new regulations were put into effect, hexachlorophene was incorporated into soaps, deodorants, body powders, vaginal sprays, lotions, cosmetics, mouthwashes, toothpastes, and liquid detergents. In most of the cosmetics it served as a preservative and was added only in very small concentrations, whereas the liquid detergents contained the highest concentrations—up to 3%. Hexachlorophene is also used as a fungicide on ornamental plants, as a mildewstat on conveyor chains, in laundry rinses, in shoes, and in air filters, and as a fungistat on leather, paper, and textiles in associated industries (3).

In soaps and deodorants it enhances the deodorizing effect by inhibiting the growth of bacteria on the skin. Because it reduces the number of bacteria, particularly gram-positive bacteria on the skin, it is also employed as a surgical scrub solution and for washing babies in newborn nurseries.

Hexachlorophene is usually classified as a bacteriostatic rather than a bacteriocidal agent, the argument being that the bacteria are not killed but that their growth is inhibited, and when exposure to the chemical is discontinued, they recover and multiply. In studies with one bacterial strain (asporogens KM strain of *Bacillus megaterium*), Silvernale and co-workers (4) found that at high concentrations hexachlorophene was bacteriocidal, but that at lower concentrations it was bacteriostatic. The same authors (5) found that treatment of bacteria with hexachlorophene resulted in leakage of intracellular solutes, derived from ribonucleic acids and protein in the cell. Gram-positive bacteria, yeast, and mold were most susceptible, whereas dormant spores, *Pseudomonas*, and paracolon strains were resistant to this effect of hexachlorophene. The gram-negative paracolon bacteria mentioned in these studies had been grown in a medium where hexachlorophene was the only added carbon source and had become highly resistant to the drug.

Similar observations have also been made in practice. Evans and co-workers (6) noted a striking increase in gram-negative bacteria on the toe webs of human volunteers after one week of using hexachlorophene soap. After 14 days, the growth had diminished. The numbers of diphtheroids and staphylococci on the skin were significantly reduced both times.

II. STAPHYLOCOCCUS INFECTIONS IN INFANTS AND USE OF HEXACHLOROPHENE ON INFANTS AND OTHER SEGMENTS OF THE GENERAL POPULATION

In the late 1950s and early 1960s epidemics of severe staphylococcal disease occurred in newborn nurseries or the disease developed in the infants after they were discharged from the hospital (7,8). These outbreaks were usually caused by strains of penicillin-resistant, coagulase-positive staphylococci of a specific phage type. In most instances the attending hospital personnel were carriers of these organisms and transmitted them to the infants, but transmission also occurred through the mother, environmental surfaces in the nursery, and air filtering systems and other equipment. In monitoring infants two very important factors had to be considered: the number of infants from whom coagulase-positive staphylococci of a specific phage type could be cultured and the number of infants who actually developed disease. The clinical manifestations of hospital-acquired infection appear predominantly within the first month of life but (at least in the United States, where hospital stays are short) often after the infant has been discharged from the hospital. Thus, the infant may also infect other members of his family. If the infant remains free of staphylococcus organisms during the hospital stay, his chances of developing a staphylococcal infection during the neonatal period after discharge from the hospital will be less likely (9). The staphylococci are usually first demonstrated in cultures of the umbilicus or the region of the groin, axilla, and abdomen; from there they spread to the nose, throat, eyes, and other parts of the body.

Success in reducing staphylococcus colonization and infection using hexachlorophene on newborn skin was reported by many in the early 1960s and has recently been reviewed by Gluck (10). In their well-controlled study, Gezon and co-workers (9) found that the incidence of staphylococcus growth in infants washed with hexachlorophene in the nursery was 24% when cultures were taken at discharge, but that the incidence was 52% for those infants who were washed only with the detergent base. A follow-up study at three weeks of age showed that 41% of the cultures from those babies who continued to be washed with hexachlorophene at home were positive and that 67% of the cultures from those washed with the detergent base were positive. Disease was observed in 3.3% of the hexachlorophene-washed infants and in 18.6% of the detergent-base-washed infants. The authors point out that they could not always reduce staphylococcal colonization, as reported in this study,

and that not all infections in the nursery can be prevented by washing with hexachlorophene. The problem may merely be converted from staphylococcal disease to infections by other pathogens. Numerous other studies have been reported in the literature describing the effectiveness of preventing staphylococcal colonization of infants with hexachlorophene (11-13). This was again emphasized by Gezon and co-workers (14) after reviewing their findings made in extensive studies over a 6-year period. These same authors also pointed out that the incidence of *Candida albicans* and *Klebsiella* infections was higher in infants not infected with staphylococci than in those infected.

Forfar and his co-workers (15) had reported similar results of infection rates in newborn nurseries at two hospitals during a 10.25-year period. In one of the hospitals where hexachlorophene was never used, the staphylococcal infection rate ranged from 2.2 to 9% and the gram-negative infection rate from 0.4 to 3.2% throughout the entire observation period. Neither of the infection rates showed any particular trend.

In the other hospital where the infection rate ranged from 8.5 to 14.3%, use of hexachlorophene resulted in a reduction of staphylococcal infections to 2.8% in the first period, but the rate later increased to 4.1% in one particular period. After the first six months of hexachlorophene use, the incidence of infections with gram-negative bacteria rose to levels much higher than had been observed before hexachlorophene was introduced. This study also showed that in nurseries where hexachlorophene was not used, a high incidence of staphylococcal infections was accompanied by a high infection rate with gram-negative bacteria, and when the infection rate for staphylococcus was low, so was the infection rate for gram-negative bacteria. When the use of hexachlorophene was introduced, staphylococcus infection rates and carrier rates were reduced, but gram-negative infection and carrier rates rose much higher. The authors concluded that this increase in gram-negative infection rates did not seem to be solely due to the reduction of staphylococcal infection.

Light and Sutherland (16) found that the epidemics of severe staphylococcal disease observed in the late 1950s and early 1960s are not occurring in present-day nurseries. These epidemics were characterized by breast abscesses, osteomyelitis, pneumonia, and other severe pyogenic lesions, and they usually resulted from infection with a very virulent staphylococcus strain, such as phage type 80/81. When these authors reviewed their surveillance cultures for the 12-year period from 1960 to 1972, they found that a highly significant decrease in the colonization of infants with virulent strains had occurred. This decrease was unrelated to the use of hexachlorophene. Although the nurses washed their hands with hexachlorophene during this entire time, the infants in the new

born nursery were washed with hexachlorophene only during a 2-year period from 1965 to 1967 and the infants in the premature nursery were never washed with hexachlorophene.

According to Light and Sutherland (16) the use of hexachlorophene does not control staphylococcal epidemics, but it will decrease colonization with this organism and reduce the incidence of endemic disease. A decrease of colonization with *Staphylococcus aureus* has also been noted to result in an increase in gram-negative bacteria, particularly *Pseudomonas*. This effect of changing the bacterial flora of the skin and other areas after repeated application is not specific to hexachlorophene, but also occurs with other antibacterial agents. It takes place not only in the newborn, but also in other segments of the general population (17-22).

A number of the papers cited above were presented at a conference on the use of hexachlorophene, at which time one of the participants (23) pointed out that staphylococcal epidemics occurred despite hexachlorophene bathing, which has not only failed to control staphylococcal outbreaks, but results in increased colonization with gram-negative organisms. Hexachlorophene bathing may, however, modestly affect nursery colonization with *Staphylococcus aureus* and may delay the appearance of staphylococcal disease. Changes in the frequency of staphylococcal disease in newborn infants occur spontaneously and are not necessarily associated with hexachlorophene-induced alteration of colonization.

At the same conference, discontinuation of hexachlorophene bathing was reported to have resulted in an increased incidence of staphylococcal infection in the newborn (24). Plueckhahn (25) reported control of nursery infections with the use of hexachlorophene applications. He also observed an increased number of gram-negative organisms on the infants. Whether any of the effectiveness of hexachlorophene could have been due to a spontaneous regression of staphylococcal disease cannot be determined from Plueckhahn's report.

Epidemiological investigations undertaken recently by Dixon and co-workers (26) showed that in 60 out of 66 confirmed outbreaks of staphylococcal disease, discontinuation of hexachlorophene bathing of newborn infants preceded the epidemic. The disease in six outbreaks was unrelated to the use or nonuse of hexachlorophene; in two the epidemic began before hexachlorophene was discontinued; and in two hexachlorophene usage persisted in mixed-pattern. A retrospective survey conducted by Kaslow and his co-workers (27) following the FDA announcement recommending curtailed use of hexachlorophene showed that in 208 hospitals staphylococcal disease increased within the 3 months after hexachlorophene bathing was discontinued. The incidence of staphylococcal disease increased from one to two cases per 1000 to about six cases per

1000 infants. However, the rate of staphylococcal disease in nurseries that had never used hexachlorophene also rose from no infections in the preceding 4 months to about three cases per 1000 admissions to the newborn nursery. Unfortunately, this group of 22 hospitals was much smaller than the 186 hospitals that had at some point used hexachlorophene. This natural fluctuation in staphylococcal disease is evident in the finding by Evans and his co-workers (28) that a natural decline of *Staphylococcus aureus* usually occurs in the fall. A 4-year survey of 1804 infants demonstrated cyclical fluctuations for *Staphylococcus aureus* and other bacteria but not for *Staphylococcus epidermis* and *Enterobacter* organisms. In this study cultures were obtained from the anterior nares and the umbilicus.

The problem of the efficacy of hexachlorophene in particular, and other topically applied bacteriostatic agents in general, is very complex, and most studies on the use of these materials lack information on follow-up after discharge from the hospital or lack adequate controls, or introduced the bacteriostatic agent in the wake of an outbreak of disease when other measures of control were instituted at the same time. Much is still to be learned about the natural fluctuations of the bacterial flora on the skin. A number of studies reported only the decrease of the staphylococcus on the skin and failed to account for other bacteria. It is generally agreed, however, that the washing of nurses' hands with a hexachlorophene-containing detergent at a concentration of 3% reduces staphylococcal infection in neonatal nurseries and that surgical hand-scrubbing reduces postsurgical wound infections.

The treatment of acne vulgaris with hexachlorophene and other bacteriostatic agents has met with mixed success and has at times resulted in overgrowth by gram-negative bacteria (17). No scientific data on the usefulness of vaginal hygiene products containing hexachlorophene or other bacteriostatic agents are available. Information of their effect on mucous membranes or the normal bacterial flora of the vulva and lower part of the vagina is also not available.

Brunn (29) investigated the frequency of side effects to hospital personnel and surgical patients produced by a hexachlorophene detergent. A significant skin irritation was observed in 35 out of 1047 patients, and in 26 of them the use of hexachlorophene had to be discontinued. Skin irritation was most frequent in the perineal region, where it occasionally took on the aspect of an acute dermatitis. Use of hexachlorophene for handwashing by the hospital personnel resulted in skin irritation and had to be stopped in 3 out of 441 persons. In most instances the irritation disappeared with regular use of hand cream. The author also observed a slight increase in the colonization of *Candida albicans* in surgical wounds and an increase in gram-negative bacteria during certain periods of the

study. Skin irritation after repeated application, particularly of a 3% hexachlorophene detergent solution, in humans has also been reported by others, especially for the scrotum (30,31).

III. TOXICITY OF HEXACHLOROPHENE IN ANIMALS

Hexachlorophene, when given orally in an appropriate vehicle, is very toxic in most animal species tested. We found that the acute oral LD_{50} was 56 mg/kg in the adult female rat, 66 mg/kg in the adult male rat, and 120 mg/kg in weanlings (32). Nieminen and co-workers (33) found an acute oral LD_{50} in 10-day-old suckling rats of 9 mg/kg bodyweight. In other animal species, the oral toxicity is of about the same order of magnitude. Gump (1) lists an acute oral LD_{50} for mice, for instance, as 168 mg/kg. The single oral LD_{50} for sheep and cattle was between 30 and 60 mg/kg (34).

Little has been reported on dermal toxicity; it was generally assumed that only small amounts of a given dose are absorbed through the intact skin. However, immediately after the infliction of a second-degree burn, the average absorption rate is increased $2\frac{1}{2}$ times in rats (35). Repeated application of hexachlorophene in either a detergent or propylene glycol solution to the shaved backs of rats caused skin irritation, which could become quite severe and resulted in sufficient absorption to produce systemic toxicity (32). When hexachlorophene was applied to the skin daily for about a month at a 3% solution in either propylene glycol or a detergent at a daily dose of 24 mg/kg bodyweight, the rats developed symptoms of leg weakness and morphological changes of the white matter of the central nervous system consistent with status spongiosus.

When rats were fed high dietary levels, such as 400 or 500 ppm of hexachlorophene, they developed leg weakness within 12 to 19 days and eventually just dragged their hind legs on the bottom of the cage (36). The wet weight of the brain markedly increased to about 30% over the weight of the control brains after a 14-week feeding period. This increase in wet weight of the brain was accompanied by an increase in water and sodium, while the electrolyte composition of the blood remained unchanged (37). The increase in the water content of the brain was also observed by Nieminen and co-workers (33). The function of the hind legs returned gradually when exposure to hexachlorophene was halted (36).

Hexachlorophene has a specific effect on the brain and spinal cord and at very high doses also on the myelinated peripheral nerves. The lesion of the central nervous system is confined to the white matter and

on light microscopic examination consists of vacuoles of varying sizes, which are in close proximity to each other and are surrounded by strands of myelin. The vacuoles appear to be empty (Fig. 2). When fairly high doses of hexachlorophene are given, the vacuolation is diffusely distributed throughout the entire white matter of the brain and spinal cord. With lower doses or shorter exposures, the vacuoles may be smaller, may be localized only in certain parts of the central nervous system, or may not be present at all or not in all animals to the same degree. The gray matter is normal. Electron microscopic examination of the brain reveals that the vacuoles are caused by a split in the myelin sheath at the intra-period line. The axon of the myelinated nerve may either be intact or pushed to one side. The vacuoles within the myelin sheath probably contain electron translucent fluid, or occasionally a few electron dense granules, and at times they are traversed by strands of myelin (36,38). Status spongiosus has also been produced in the rat's myelinated peripheral nerve with very high doses of hexachlorophene and alterations of the node of Ranvier, and decreased conduction velocities have been re-

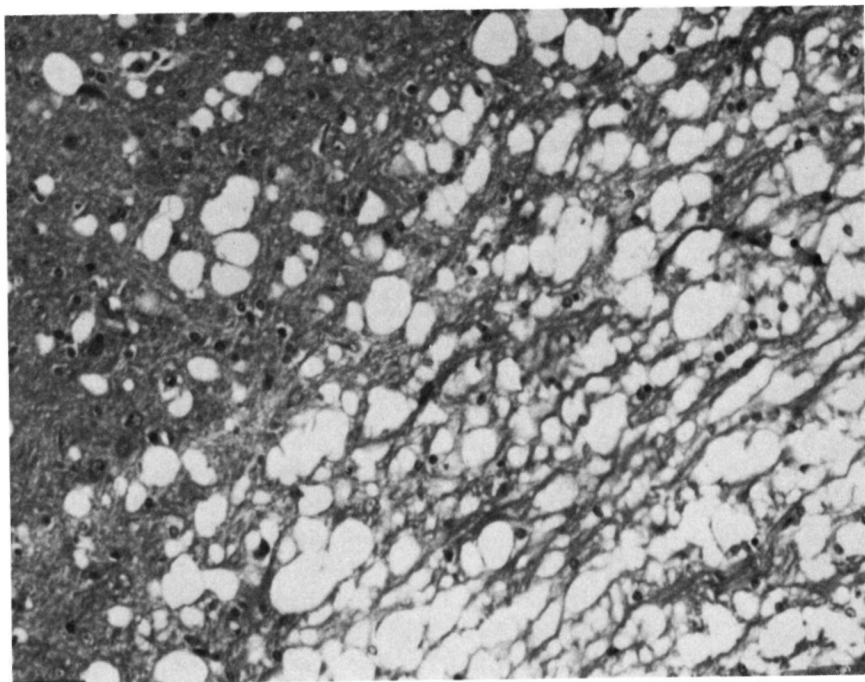


FIG. 2. Section of rabbit cerebrum showing moderate vacuolation of the white matter, H&E $\times 100$.

corded (39,40). In sheep, hexachlorophene particularly affects the optic nerve, where the lesion is irreversible (34). Since the brain lesion was first described in the rat, it has been induced in a number of other animal species: monkeys, sheep, mice, rabbits, cats, and tadpoles (38,41). The gray matter is not affected in any of these animals. Although in the adult rat several doses of hexachlorophene must be given before the brain lesion becomes manifest, in the young weanling rat a single dose causes some alterations in the white matter (42).

In the suckling rat the brain lesion cannot be produced during the first 8–10 days of life. During this time the rat brain does not contain any myelin. However, when myelin is laid down, which in the rat occurs most rapidly between 12 and 25 days of age (43), the sucklings become very susceptible to hexachlorophene and develop symptoms of neurotoxicity, which present themselves primarily as tremor and impairment of front limb function rather than hind limb function as we know it in the adult rat (44).

When rats were given hexachlorophene during the suckling period, a decrease in the yield of purified myelin was noted, but the composition of myelin was normal. During the purification of myelin from rats given hexachlorophene an abnormal "floating fraction" was observed with a chemical composition that differed in some respects from that of normal myelin. The presence of a "floating fraction" is considered to indicate myelin degradation (45).

When 22- or 60-day-old rats were started on the same dosing regimen as the sucklings, they did not develop signs of neurotoxicity or status spongiosus of the white matter of the brain. During the period when the rats were most susceptible to the neurotoxic effect of hexachlorophene, hexachlorophene brain levels, as well as blood levels, were much higher than in the postweaning period (44). Several factors may play a role in producing these higher levels in the brain and, in part, also the higher levels in the blood. Hexachlorophene absorption from the gastrointestinal tract may be increased during this period and may be less well metabolized in the liver; excretion may be less efficient; it may be less well bound to blood albumin, and it may penetrate into the brain more easily because at this time the blood brain barrier is not well developed.

At least in some animal species a number of other chemicals also produce vacuolation of the white matter of the brain (status spongiosus) (46–49), namely, triethyltin, isoniazid, 2-acetoxy-4-chloro-3,5-diiodobenzanilide and rafoxanide, which is 3,5-diiodo-3'-chloro-4'-(*p*-chlorophenoxy) salicylanilide. Normal brain tissue, particularly that of rodents, may on light microscopic examination show small vacuoles that represent an artifact (50). These artifactual vacuoles are smaller and usually

very uniform, and at times contain a homogeneous pink staining material. They are separated by wide areas of normal-appearing white matter. Brains with this artifact usually do not weigh more than those without it.

Webster and co-workers (41) were able to observe the hexachlorophene-induced vacuolation in the unfixed optic nerve of the tadpole by phase contrast microscopy, and Lampert and his co-workers (51) demonstrated it in the brains of experimental animals that were quenched in liquid nitrogen and subsequently sectioned in the frozen state. These observations illustrate that the vacuolation is not a fixation artifact.

The morphological changes observed in the brain at times do not correlate well with clinical symptoms of neurotoxicity. Apparently some of the observed symptoms are caused by intracranial pressure and perhaps pressure on the nerve roots because of the increasing size of the brain and spinal cord due to fluid accumulation. Hanig and co-workers (52) demonstrated this. When spinal punctures were performed in cats with hexachlorophene-induced paralysis, spinal fluid pressure was found to be elevated, and the slow intravenous injection of 30% urea in 10% invert sugar lowered it for several hours before it recurred. Cats that had previously been paralyzed were able to stand during the treatment period. Changes in neuromuscular function develop in rats before peripheral neuropathy becomes demonstrable. This was tested in rats with the rotating-rod test. At neurotoxic doses heat perception also was impaired (53).

No morphological changes in other organs aside from those in the nervous system have been reported in most of the published studies. High doses of hexachlorophene, introduced into the vagina of pregnant rats, will produce hydrocephalus in the rat fetus. There is also an increase in gram-negative bacteria in the vagina associated with maternal vaginal infections (54).

IV. HUMAN TOXICITY

A number of human poisoning cases, some of them fatal, have been reported. Toxicity in humans has resulted either from ingestion of several ounces of a 3% hexachlorophene solution in a detergent, usually mistaken for milk of magnesia, or from dermal exposure when hexachlorophene was applied to large areas of burned or otherwise damaged skin or to abnormal skin (1,55,56). After ingestion of toxic doses of hexachlorophene, gastrointestinal symptoms such as nausea, vomiting, and diarrhea develop first. These are followed by signs of peripheral circulatory failure; body temperature fluctuations and central nervous system symptoms may develop, manifested only as headaches, or also in diplopia, nystagmus, muscle twitching, and convulsions (37). If poisoning occurs

through skin absorption, gastrointestinal symptoms are not as prevalent, although anorexia and vomiting may occur. Children poisoned by hexachlorophene may become what has been termed "behavior problems," that is, they become uncooperative, lethargic, and withdrawn (57), show various neurological deficits, and may have convulsions.

Mullick (57) studied the brains of four children who died following three or more days of exposure to a detergent containing 3% hexachlorophene. Two of these children had congenital ichthyosis, an abnormal skin condition, and two had had fairly extensive burns. The burns were healing well, however, when the children died. All four children showed status spongiosus of the white matter similar to that observed in experimental animals.

Shuman and his co-workers (58), in a retrospective blind study, demonstrated that the incidence of status spongiosus in the brainstem reticular formation in infants who had died from a variety of causes could be related to hexachlorophene exposure. The authors found 21 cases in a series of 250 autopsies. Of these, 18 were infants of less than 30-weeks' gestation and weighed less than 1400 gm. All but two of the 18 infants had received three or more total washes with a 3% hexachlorophene detergent solution. Among the cases studied, a group of stillborns did not show the lesions and, of course, had not been washed with hexachlorophene. Shuman and his colleagues (58) also mentioned earlier cases of Letterer-Siwe's disease in which they had observed status spongiosus. These children had had extensive exposure to hexachlorophene. Powell and his co-workers (59) found that seven out of thirteen infants with four or more exposures to a 3% hexachlorophene solution and weighing less than 1400 gm showed status spongiosus of the myelinated tracts of the brain stem. Electron microscopic examination of these lesions showed that the spongy change observed in the premature infants was due to a split in the myelin sheath.

In August 1972, the deaths of a number of infants in France were reported in the press. The cause of this poisoning outbreak was a baby powder to which hexachlorophene had inadvertently been added, resulting in a final concentration of 6% hexachlorophene in the powder. Normally, this baby powder did not contain hexachlorophene. Forty-one infants and small children eventually died over a 4-month period, from April to August, and many more were sick but recovered (60). Electroencephalographic changes were reported in a few of these cases (61).

The French episode has not been reported in full in the scientific literature since many civil court cases are pending, and until these litigation problems have been settled, the total number of cases is not public information.

These various reports indicate that the infant and young child in par-

ticular will, with ample exposure, develop brain lesions like those observed in experimental animals. Again, these brains have to be evaluated for status spongiosus against a background of artifacts. It is also very important, particularly in the premature infant, that the brainstem be studied, since in many areas of the brain of the premature infant myelin is not present.

Status spongiosus of the white matter of the brain, as far as we know, represents a specific or restricted type of brain edema, with the fluid accumulation confined to the myelin sheath. The term status spongiosus, or spongy change, is a descriptive term, and this morphological alteration is not unique for the white matter. Status spongiosus either of the white or gray matter and either localized or diffuse has been reported in conjunction with a variety of diseases (47,62,63). From our own observations and those reported by others, it can be concluded that this brain lesion is reversible upon removal of the cause; however, morphological changes disappear only slowly, and as the incident of the sheep exposures illustrates, not always completely (34). In 1-year feeding studies of rats with dietary levels of hexachlorophene sufficient to produce the brain lesion, no additional morphological changes of the brain other than status spongiosus were observed (42).

V. SKIN ABSORPTION, HEXACHLOROPHENE BLOOD LEVELS, AND TISSUE LEVELS

Depending on solubility, vapor pressure, and physical characteristics most chemicals can, to varying degrees, penetrate the human skin. Because of several layers of epithelial cells, as well as the keratinous layer, penetration occurs at a very low rate for many substances. To be absorbed through the skin, a chemical must be able to penetrate the cell membrane. Most cell membranes consist of 40% lipid and 60% protein. The lipid portion is predominantly composed of polar lipids (phosphoglycerides, sphingolipids, glycolipids) of different types. Almost all membranes are freely permeable to water and to neutral lipophilic materials. According to the unit membrane hypothesis, a membrane consists of a continuous bilayer of mixed polar lipids, and each surface of this layer is coated by a monomolecular layer of protein molecules. Nonpolar molecules, and particularly those that are lipid soluble, dissolve readily in this bilayer of lipid membranes (64).

After applying hexachlorophene preparations to the skin, a residue remains even after rinsing (65). This hexachlorophene residue probably attaches itself to proteins. From observations made by nuclear magnetic resonance spectrometry, Hague and Buhler (66) suggest an intermolecu-

lar interaction between the phenolic protons and the oxygen atoms of amides and related compounds, which results in hydrogen bonds between the phenolic proton of hexachlorophene and the carbonyl oxygen of the polypeptide. Subsequently, hexachlorophene is gradually absorbed from this pool of material remaining on the skin and enters cells fairly easily because it is soluble in the bilayer of lipid membranes.

Brown and co-workers (67) in *in vitro* studies found that the greatest penetration of hexachlorophene through the stratum corneum of human skin occurred when the receptor fluid consisted of 0.2 *M* sodium phosphate buffer to which 3% albumin was added and Ringer's solution was used as the diluent. The rate was 22 ng/cm²/hour for the first 24 hours, and then it was absorbed at a lower rate for the next 48 hours. If hexachlorophene alters the keratinous layer of the skin in any way, then this would also affect absorption. Other factors to be considered in skin absorption are skin hyperemia, alteration in skin temperature, and hydration. The skin of the premature infant, for instance, is extraordinarily permeable, since its texture differs from that of the term infant and older child (68).

A number of studies have been reported in which blood levels of hexachlorophene were determined in adults as well as in infants. Several methods for the determination of blood levels and other tissue levels are available. The various advantages and disadvantages of these methods are discussed by Ulsamer (69). Depending on the type of solvent used for extraction of hexachlorophene from blood and tissues, the recovery may vary from 60 to 95%. This, among other things, may explain some of the variations observed in the blood levels reported from different laboratories for subjects with the same exposure. Many of the blood levels reported are for whole blood. When hexachlorophene is determined in plasma, levels are higher, since hexachlorophene is mostly bound or attached to albumin and only very little to the cell membranes of mammalian red blood cells, (92% in adults, 87% in newborns, and 82% in rats). Frog and fish albumin only bind 19 and 5%, respectively (70). Hexachlorophene is normally conjugated to the glucuronide by the liver and excreted in the bile (71). Thus far, no studies have been reported in which the total amount of hexachlorophene, bound and unbound, as well as possible metabolites, was accounted for with radioactive material in the bloodstream and correlated to measurements undertaken with gas chromatography. Information from such studies would provide a better understanding of what is being measured by gas chromatography. At present, it is assumed from experience with other chemicals that only the unconjugated hexachlorophene is determined in the bloodstream by gas-liquid chromatography.

For some drugs such as diphenylhydantoin, the unbound fraction of

the drug may be higher in hyperbilirubinemic infants than in infants with lower bilirubin levels. Various drugs and antiseptics may compete for binding sites, some of them replacing bilirubin. Furthermore, the formation of glucuronides may be quite variable in the newborn and often much lower than in the adult (72). Moreover, premature infants have no glucuronyl transferase, which is necessary for the formation of glucuronides (73), and a small percentage of the general population suffers from a genetic deficiency of glucuronyltransferase (74). In the premature and the term infants, detoxification in the liver, as well as excretion, may not be as efficient as in the normal adult. Gandolfi and Buhler (75) found that extensive enterohepatic circulation of hexachlorophene occurs in the rat, and ligation of the bile duct increases the toxicity of hexachlorophene. All of these factors, in addition to differences in skin absorption, could account for higher blood levels of hexachlorophene in neonates, children, and some other individuals under similar conditions of exposure.

A number of studies, both published and unpublished, have been conducted in various population groups to determine hexachlorophene blood levels. In our own, we found a mean concentration of 0.022 ppm in the cord blood of infants. After repeated washings with hexachlorophene, these same infants at discharge from the hospital showed a mean concentration of 0.109 ppm in whole blood. In the blood of 14 adults from the general population the mean level of hexachlorophene concentration was 0.028 ppm (76). In comparison, rats with very severe brain lesions that had been fed hexachlorophene at a rate of 500 ppm had blood levels of 8.5 ppm and those with only occasional focal brain lesions in the central nervous system that had received 100 ppm had blood levels of 1.21 ppm (37). Other investigators found that when five infant monkeys were washed daily with a 3% hexachlorophene detergent solution, the plasma levels of hexachlorophene were 3.1 ppm after one week and somewhat lower at 3 months, namely 2.3 ppm. Since it is currently assumed that plasma levels are twice as high as whole blood levels, this would compare to whole blood levels of 1.5 and 1.1 ppm, respectively. When killed after daily washing for 3 months, these monkeys showed status spongiosus of the white matter of the central nervous system identical to the lesion described in rats (77).

Blood levels in infants reported by others following the use of hexachlorophene were of the same order of magnitude as those reported by us (38). The fact that hexachlorophene was also found in cord blood suggests transplacental passage (76).

Premature infants after washing with 3% hexachlorophene solutions have higher hexachlorophene blood levels, sometimes reaching values of

over 1 ppm (38,78). This may well represent increased absorption and a slower metabolism of hexachlorophene once it is absorbed.

A number of blood level determinations have also been conducted in adults exposed to hexachlorophene either by repeated hand-washing or bathing or by simulated surgical scrubs (77). The highest mean blood level after surgical scrubs was 0.1 ppm, while daily total body bathing with a 0.75% hexachlorophene cake soap for 21 days resulted in blood levels in males of 0.52 and in females of 0.28 ppm, with maximum concentrations of 0.73 and 0.42 ppm, respectively. In another study of levels of hexachlorophene in the blood of operating room personnel, scrubbing with 3% hexachlorophene detergent solution three times a day for 21 days resulted in blood levels of 0.2 ppm. Preparations with lower concentrations of hexachlorophene produced lower blood levels (79). However, preparations containing low concentrations of hexachlorophene may be less effective in preventing infection.

From data obtained primarily in animal studies, it has been assumed that levels above 1.5 ppm in whole blood should be considered toxic. Although all findings indicate that hexachlorophene is eliminated from the body fairly rapidly, results of extensive pharmacodynamic studies with hexachlorophene have not been published. When hexachlorophene blood levels were measured by gas-liquid chromatography in the rabbit following a single dermal dose, the blood level peaked within 7 hours (80). After a single dermal dose, the time interval between application of hexachlorophene and obtaining blood is quite important. Following repeated dermal applications of hexachlorophene, this is probably less important, since a deposit is built up on the skin from which the blood levels are constantly replenished, and the hexachlorophene blood level is believed to plateau in relation to the amount present on the skin.

As far as toxicity is concerned, the hexachlorophene level in the brain is more important than the blood level. Adult squirrel monkeys with mild status spongiosus of the white matter of the brain showed brain levels of 0.38 ppm 24 hours after the last hexachlorophene dose; the determinations were made by gas-liquid chromatography and the recovery rate was 75% (38). Thirteen-day-old suckling rats with severe symptoms of neurotoxicity 4-6 hours after the last of three daily doses of 10 mg/kg hexachlorophene by stomach tube had hexachlorophene blood levels of 26.4 and brain levels of 10.0 ppm, whereas in other rats 13-15 days old at sacrifice, 22-24 hours after the last of three daily doses of 10 mg/kg of hexachlorophene the levels were 4.3 and 3.9 ppm, respectively (44). Neither at an age of 4-8 days nor when the rats were 20-27 days old were such high levels found in the brain with the same dosing regimen. Thus,

when hexachlorophene is most toxic to the rat, it also seems to enter the brain most easily. Whether toxic and lethal brain levels for the adult animal are the same as for the very young animal and whether species differences exist in this respect has not been sufficiently studied.

VI. OTHER TOXIC EFFECTS OF HEXACHLOROPHENE

Like other substituted phenols, hexachlorophene uncouples oxidative phosphorylation (81,82). However, Harris and co-workers (83) were unable to confirm the uncoupling effect of hexachlorophene in the rat brain in *in vivo* studies. It also inhibits succinoxidase activity (84). In connection with the uncoupling of the oxidative phosphorylation, Nakaue and co-workers (85) also observed that the body temperature of rats became elevated following toxic doses of hexachlorophene and such rats developed a very rapid and very pronounced *rigor mortis* when they were sacrificed (37). Hepatotoxicity with morphological alterations, mainly periportal lipid accumulation, was reported in sheep by Pugh and Crowley (86) and also by Thorpe (87). We were not able to demonstrate any morphological alterations in the livers of rats fed hexachlorophene and examination of the rat liver by electron microscope did not reveal any ultrastructural changes. Since, as was pointed out earlier, hexachlorophene is made from trichlorophenol, the possibility always exists that hexachlorophene may be contaminated with a chlorinated dibenzodioxin, mainly 2,3,7,8-tetrachlorodibenzodioxin, which for many animals is a very potent hepatotoxin, and this may explain the findings in the sheep. Of course, the finding may also represent a species difference in response. We were never able to demonstrate chemically a chlorinated dibenzodioxin in the product we used in our studies. Furthermore, the brain lesion that develops after exposure to hexachlorophene has not been produced by 2,3,7,8-tetrachlorodibenzodioxin; neither has this product ever been shown to produce the type of paralysis observed in the rats fed hexachlorophene. Chloracne, on the other hand, which is the typical skin lesion in humans that develops after exposure to tetrachlorodibenzodioxin, has never been observed in connection with the use of hexachlorophene. We can therefore safely assume that contamination of the marketed hexachlorophene with tetrachlorodibenzodioxin generally does not present a problem. Of course, if anything were to go wrong in the production process or good quality control were not exercised, this could result in the contamination of hexachlorophene with 2,3,7,8-tetrachlorodibenzodioxin and in the development of chloracne, hyperkeratosis, hyperpigmentation, liver disease, and a number of other toxic effects in the user of such a product.

By feeding various dietary levels of hexachlorophene to rats in the course of a reproduction study, we found that the survival of the pups was reduced in comparison to the controls when the dams were fed dietary levels of 100 ppm, which corresponds to a dietary intake of 11.8–5.5 mg/kg bodyweight/day. Since hexachlorophene is also excreted in milk, this may have contributed to the toxic effect of the product in the offspring. As pointed out earlier, the sucklings are much more susceptible than adult animals to the toxic effects of hexachlorophene (32). The pups, as they approach weaning, may also consume some of the diet intended for the dams, which of course would increase the exposure. In this same study a dietary level of 20 ppm (2.3–1.1 mg/kg bodyweight/day) did not affect the survival of the offspring, nor did the administration of fairly high doses of hexachlorophene to the dams during pregnancy (32) produce any effect on the offspring.

VII. LEGAL RESTRICTIONS OF HEXACHLOROPHENE

Because of the long and apparently safe experience with the topical use of hexachlorophene, no specific safety evaluation of the product was undertaken before it was used in newborn nurseries or on burned patients. Following the report that the dermal application of hexachlorophene on the skin of newborn monkeys (77) resulted in the same brain lesion that had been produced by feeding studies with rats (36), the FDA in mid-December 1971 issued a "Drug Bulletin" to all physicians, summarizing these studies and pointing out that they challenged the safety of hexachlorophene.

On January 7, 1972, a statement concerning the use of hexachlorophene in cosmetic and drug products was published in the *Federal Register*. The FDA proposed that hexachlorophene be removed from all cosmetics, except when it was present at very low levels (0.1%) as part of a preservative system. "New Drug Applications" were to be required for all drugs containing hexachlorophene, and drugs containing more than 0.75% hexachlorophene had to bear the prescription label. A 60-day period was given for comments on these proposals (60). During this time, many hospitals apparently curtailed the use of hexachlorophene in newborn nurseries. Subsequent reports of increased staphylococcal infections have already been discussed in connection with the surveys (26,27) conducted by the Center for Disease Control. Following the poisoning outbreak in France and the findings of Shuman and co-workers (58) and Lampert and co-workers (51) a final notice was published in the *Federal Register* of September 27, 1972, restricting hexachlorophene to prescription use as a bacteriostatic skin cleanser and for the control of outbreaks

caused by gram-positive organisms when other procedures are unsuccessful. Hexachlorophene was banned from cosmetics and drugs sold over the counter, except when used in concentrations less than 0.1% as a preservative and only when no alternative preservative was available.

In making these decisions, the FDA was faced with several problems. First, the efficacy for many claims made for the action of hexachlorophene had never been established by present-day standards. The use of the product had proliferated so that without trying very hard the consumer could daily encounter innumerable products that contained hexachlorophene. It was known that total body bathing with hexachlorophene could result in significant absorption of the product even through the intact skin and the margin of safety between toxic and nontoxic blood levels appeared to be very narrow, leaving very little margin for conditions possibly causing increased individual susceptibility, such as age, impaired liver or kidney function, skin diseases such as eczema, neurodermatitis, ichthiosis, and other skin changes that might affect the skin barrier. On the other hand, hexachlorophene used locally on a small infection or for hand and forearm washing or similar local uses appeared safe. However, for all practical purposes, use of over-the-counter products cannot be controlled. The FDA's only means of restricting a drug is to make it a prescription drug or to ban it completely. Since no real benefit was demonstrated from the use of hexachlorophene as an ingredient in many products and since the margin of safety was narrow when it was used extensively, the FDA decided to make it a prescription drug even though its local use would in many instances have been perfectly safe. The efficacy of the use of hexachlorophene in feminine hygiene sprays, in toothpastes, for acne, for irrigating wounds, and for treatment of chronic eczema had never been established. Instead of letting the consumer decide the so-called benefit versus risk ratio, the FDA put the burden of this decision on the physician.

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